Progressive chronic heart failure slows the recovery of microvascular O2 pressures after contractions in the rat spinotrapezius muscle

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Copp SW, Hirai DM, Ferreira LF, Poole DC, Musch TI. Progressive chronic heart failure slows the recovery of microvascular O2 pressures after contractions in the rat spinotrapezius muscle. Am J Physiol Heart Circ Physiol 299: H1755–H1761, 2010. First published September 3, 2010; doi:10.1152/ajpheart.00590.2010.—Chronic heart failure (CHF) induces muscle fiber-type specific alterations in skeletal muscle O2 delivery and utilization during metabolic transitions. As a result, the recovery of microvascular PO2 (PmvO2) is prolonged in slow-twitch skeletal muscle but not fast-twitch skeletal muscle in rats with CHF. We tested the hypothesis that CHF slows PmvO2 recovery in rat skeletal muscle of a mixed fiber-type analogous to human locomotory muscles and that the degree of slowing correlates with central indexes of heart failure. Healthy control [n = 6, left ventricular end-diastolic pressure (LVEDP): 10 ± 1 mmHg], moderate CHF (n = 6, LVEDP: 18 ± 2 mmHg), and severe CHF (n = 4, LVEDP: 34 ± 2 mmHg) female Sprague-Dawley rats had their right spinotrapezius muscles (41% type I, 7% type IIA, and 52% type IIb and d/x) exposed, and PmvO2 was measured via phosphorescence quenching during 180 s of recovery from 180 s of electrically induced twitch contractions (1 Hz, 4–6 V). CHF progressively slowed the mean response time (MRT; the time to reach 63% of the overall dynamic response) of PmvO2 recovery (MRT0.63%: control: 60.2 ± 6.9, moderate CHF: 72.8 ± 6.6, and severe CHF: 109.8 ± 6.6 s, P < 0.05 for all). MRT0.63% correlated positively with central hemodynamic (LVEDP: r = 0.76, P < 0.01) and morphological (right ventricle-to-body weight ratio: r = 0.74, P < 0.01; and lung weight-to-body weight ratio: r = 0.79, P < 0.01) indexes of heart failure. The present investigation suggests that slowed PmvO2 kinetics during recovery in CHF constitutes a mechanistic link between impaired circulatory and metabolic recovery after contractions in CHF.

skeletal muscle; oxygen delivery; myocardial infarction; left ventricular end-diastolic pressure; phosphorescence quenching; partial pressure of oxygen

CHRONIC HEART FAILURE (CHF) is a complex clinical syndrome hallmarkied by impaired exercise and work capacity, particularly during the repetitive activities of daily living. Experimental evidence supports that CHF induces central and peripheral derangements that reduce O2 availability to working muscle(s) at the onset and during recovery from exercise (8, 43, 50), which mechanistically may underlie the reduced exercise tolerance. For example, given its regulatory role in skeletal muscle metabolism (22), decreases in O2 availability increase the reliance on "anaerobic energy" during (40, 48) and prolong metabolic recovery after (9, 37, 48) exercise.

While CHF-related research has focused primarily on altered rest-exercise transitions, less is known regarding the mechanisms underlying the altered off-transient responses (for review, see Ref. 25). This information is of great clinical utility given that the recovery kinetics of pulmonary O2 utilization (VO2) are more reproducible (23) and may be determined with a higher degree of fidelity (26) than onset kinetics in CHF patients. Moreover, at similar relative exercise intensities, CHF-induced alterations in off-transient pulmonary VO2 responses may exist despite similar on-transient responses compared with healthy controls (45). These data support that altered off-transient responses constitute a powerful prognostic indicator of functional capacity (10), and, therefore, analysis of the mechanistic bases of the slowed pulmonary VO2 recovery is warranted.

Our laboratory has previously demonstrated that progressive CHF increasingly slows the recovery of microvascular PO2 [PmvO2; which reflects the microvascular O2 delivery (QO2) to-VO2 balance] from contractions in rat slow-twitch soleus muscle (84% type I, 7% type IIA, and 9% type IIb and d/x) but not fast-twitch peroneal muscle [14% type I, 19% type IIA, and 67% type IIb and d/x, (11)] compared with healthy controls (34). This is important because a slow PmvO2 recovery lowers the pressure head for capillary-myocyte O2 transfer according to Fick’s law of diffusion and impairs the recovery of the intracellular metabolic milieu, thereby increasing muscle fatigue during subsequent contraction bouts (27). While these previous results have provided important mechanistic insights into the divergent effects of CHF on the recovery of PmvO2 in muscles of contrasting fiber types, it is not known whether the recovery of PmvO2 is slowed in muscle more representative of human locomotor muscle. In this regard, the rat spinotrapezius muscle possesses a mixed-fiber type composition [41% type I, 7% type IIA, and 52% type IIb and d/x, (11)] and oxidative capacity [140 ± 1.6 μmol·min⁻¹·g⁻¹ (11)] similar to the human quadriceps muscle [citrate synthase: 12.3 ± 0.3 μmol·min⁻¹·g⁻¹ (28); fiber type composition: 40% type I and 60% type II (46)], thus providing a powerful and highly relevant experimental model in which to investigate the effects of CHF on the skeletal muscle microvasculature (and potential therapeutic treatments thereof). Resolution of the effects of CHF on the dynamics of spinotrapezius muscle PmvO2 during recovery would provide crucial mechanistic insights into CHF-induced exercise intolerance and, importantly, provide a platform for future evaluations of therapeutic treatments.

Therefore, the purpose of the present study was to examine the effects of CHF on the kinetics of PmvO2 during recovery from contractions of the mixed fiber-type rat spinotrapezius muscle. We tested the following hypotheses: 1) progression to moderate and severe CHF after myocardial infarction is accompanied by a slowing of PmvO2 recovery compared with healthy controls, 2) the degree of slowing of PmvO2 off-kinetics correlates positively with indexes of central cardiovascular...
impairment, and 3) alterations in the off-transient \( P_{mvO_2} \) profile would be present irrespective of any alterations in the on-transient.

**METHODS**

Sixteen female Sprague-Dawley rats (312 ± 7 g) were studied in the present investigation. Rats were divided into the following groups: healthy controls (control group; \( n = 6 \)) and rats that received a myocardial infarction (CHF group; \( n = 10 \)). These 16 animals represent a subset of rats from a larger cohort (15) in which an off-transient response was successfully collected subsequent to measurements of \( P_{mvO_2} \) during contractions. Off-transient determination was precluded in the other animals due to technical issues (i.e., movement of the measurement plane during contractions). All rats were housed at Kansas State University with food and water provided ad libitum. All procedures and protocols described herein were approved by the Institutional Animal Care and Use Committee and were conducted according to guidelines set forth by the National Institutes of Health.

**Surgical procedures.** Myocardial infarction was induced in CHF rats via ligation of the left main coronary artery (36). Initially, CHF rats were anesthetized with a 5% isoflurane-\( O_2 \) mixture and intubated for mechanical ventilation with a rodent respirator (model 680, Harvard Instruments, Holliston, MA) for the duration of the infarction procedure. The heart was accessed through a left thoracotomy in the fifth intercostal space. The left main coronary artery was ligated with 6-0 silk suture ~1–2 mm distal to the edge of the left atrium. The final experimental protocol was initiated 7–10 wk after surgery. Given there were no differences in cardiovascular responses between sham-operated and nonsham-operated controls (47), animals in the control group were not subjected to any surgical procedure.

For the final experimental protocol, control and CHF rats were anesthetized with pentobarbital sodium (50 mg/kg administered intraperitoneally to effect), monitored continuously via toe-pinch and blink reflexes with anesthesia supplemented as necessary, and placed on a heating pad to maintain core temperature at 38°C (measured via a rectal probe). The carotid artery was cannulated, and a 2-Fr catheter-tipped pressure transducer (Millar Instruments, Houston, TX) was advanced into the left ventricle (LV) for measurements of systolic and diastolic pressures. Upon completion of the measurements, the transducer was removed, and the carotid artery was recannulated with a catheter (polyethylene-50, Intra-Medic polyethylene tubing, Clay Adams Brand, Sparks, MD) for the measurement of mean arterial pressure (MAP), infusion of the phosphorescent probe, and arterial blood sampling. The overlining skin and fascia were then removed carefully from the mid-dorsal-caudal region of each rat, and the right splanchnicus muscle was exposed in a manner that ensured the integrity of the vascular and neural supply to the muscle (2). Silver wire electrodes were then sutured (6-0 silk) to the rostral (cathode) and caudal (anode) regions of the muscle. The exposed splanchnicus muscle was continuously superfused with warmed (38°C) Krebs-Henseleit bicarbonate-buffered solution equilibrated with 5% \( \text{CO}_2 \)–95% \( \text{N}_2 \), and the surrounding exposed tissue was covered with Saran Wrap (Dow Industries, Indianapolis, IN).

**Experimental protocol.** The experimental protocol was exactly as previously described (15) with the present analysis reflecting the recovery of \( P_{mvO_2} \) from an electrically induced contraction bout of the splanchnicus muscle after locally superfused physiological Krebs-Henseleit solution. Initially, the phosphorescent probe palladium meso-tetra(4-carboxyphenyl)porphyrin dendrimer (R2; 15–20 mg/kg dissolved in 0.4 ml saline) was infused via the carotid artery catheter. After a brief stabilization period (10–15 min), the common end of the light guide of a frequency domain phosphorimeter (PMOD 1000, Oxygen Enterprises, Philadelphia, PA) was positioned ~2–4 mm superficial to the dorsal surface of the exposed right splanchnicus muscle over a randomly selected muscle field absent of large vessels. This region contained principally capillary blood, and \( P_{mvO_2} \) was continuously measured via phosphorescence quenching (see below) and reported at 2-s intervals throughout the duration of the 180-s contraction protocol (1 Hz, 4–6 V, 2-ms pulse durations) elicited via a Grass stimulator (model 888, Quincy, MA) and for a minimum of 180 s during the immediate postcontraction recovery period. After recovery, an arterial blood sample was drawn from the carotid artery catheter, the animal was killed by a pentobarbital sodium overdose, the thorax was opened, and the lungs and heart were removed. The right ventricle (RV) was separated from the LV and right atria, and both the lungs and RV were weighed to obtain the RV weight-to-body weight and lung weight-to-body weight ratios. Pfeffer et al. (41) demonstrated that the RV weight-to-body weight ratio is a better indicator of ventricular dysfunction than the LV weight-to-body weight ratio.

### \( P_{mvO_2} \) measurement and curve-fitting.

The principle of the phosphorescence quenching technique have been previously described (3, 5, 29, 33, 44). Briefly, the Stern-Volmer relationship permits the calculation of \( P_{mvO_2} \) through the direct measurement of a phosphorescence lifetime via the following equation described by Rumsey et al. (44):

\[
P_{mvO_2} = \left( \frac{(\tau_0^2)}{\tau_0^2 - \tau^2} \right) \left( \frac{k_q \times \tau^2}{k_0^2 \times \tau^2} \right)
\]

where \( k_q \) is the quenching constant (expressed in mmHg\(^{-1}\)s\(^{-1}\)) and \( \tau_0 \) and \( \tau \) are the phosphorescence lifetimes in the absence of \( \text{O}_2 \) and the ambient \( \text{O}_2 \) concentration, respectively. For \( R_2 \), \( k_q \) is 409 mmHg\(^{-1}\)s\(^{-1}\) and \( \tau_0 \) is 601 µs (29); these characteristics do not change over the physiological range of \( \text{pH} \) and temperature in the rat in vivo, and, therefore, the phosphorescence lifetime is solely affected by \( \text{O}_2 \) pressure (29, 44). The R2 probe binds to albumin and is thus uniformly distributed throughout the plasma. This characteristic ensures that \( R_2 \) remains within the microvascular space and does not filter into the surrounding tissue, thus ensuring the valid measurement of \( P_{mvO_2} \) (42).

Curve fitting of the measured \( P_{mvO_2} \) was performed with commercially available software (SigmaPlot 9.01, Systat Software, San Jose, CA), and the data were fit with either a one- or two-component model as described below:

**One component:**

\[
P_{mvO_2} = \frac{\Delta P_{mvO_2} \times (1 - e^{-T_o/T})}{e^{T_o/T}}
\]

**Two component:**

\[
P_{mvO_2} = \frac{\Delta_1 P_{mvO_2} \times (1 - e^{-T_1/T_1})}{e^{T_1/T_1}} + \frac{\Delta_2 P_{mvO_2} \times (1 - e^{-T_2/T_2})}{e^{T_2/T_2}}
\]

where \( \Delta P_{mvO_2} \) is \( P_{mvO_2} \) at any given time \( t \), \( \Delta P_{mvO_2(contr)} \) is the prerecovery contracting steady state as determined from average \( P_{mvO_2} \) over the last ~15 s of contractions, \( \Delta_1 \) and \( \Delta_2 \) are the amplitudes for the first and second components, respectively, \( T_1 \) and \( T_2 \) are the time delays for each component, and \( \tau_1 \) and \( \tau_2 \) are the time constants (i.e., time to 63% of the final response value) for each component. Goodness of fit (including, specifically, the selection of a one- or two-component model; see Ref. 33 for more detail) was determined using the following criteria: 1) the coefficient of determination \( (r^2) \), 2) sum of the squared residuals, and 3) visual inspection and analysis of the model fits to the data. The mean response time \( (\text{MRT}; \text{a parameter describing the overall dynamic response}) \) of the recovery period \( (\text{MRT}_{\text{off}}) \) was calculated to provide an index of the overall kinetic response according to the following equations (31):

**One-component response:**

\[
\text{MRT}_{\text{off}} = \text{TD} + \tau
\]

**Two-component response:**

\[
\text{MRT}_{\text{off}} = \left( \frac{\Delta_1}{\Delta_{\text{tot}}} \right) \left( \frac{\text{TD} \times \tau_1}{\tau_1} \right) + \left( \frac{\Delta_2}{\Delta_{\text{tot}}} \right) \left( \frac{\text{TD} \times \tau_2}{\tau_2} \right)
\]

where \( \Delta_{\text{tot}} \) is the total amplitude of \( P_{mvO_2} \) during recovery (i.e., \( \Delta_1 + \Delta_2 \)). Additionally, the time taken to reach 63% of the final response \( P_{mvO_2} \) was determined independently from the modeling procedures.
Table 1. Morphological and hemodynamic characteristics and MAP of healthy control, moderate CHF, and severe CHF rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Moderate CHF</th>
<th>Severe CHF</th>
</tr>
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<tbody>
<tr>
<td>Left ventricular end-diastolic pressure, mmHg</td>
<td>10 ± 1</td>
<td>18 ± 2*</td>
<td>34 ± 2*†</td>
</tr>
<tr>
<td>Right ventricle-to-body weight ratio, mg/g</td>
<td>0.58 ± 0.02</td>
<td>0.69 ± 0.03*</td>
<td>1.23 ± 0.08*†</td>
</tr>
<tr>
<td>Lung weight-to-body weight ratio, mg/g</td>
<td>3.9 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>9.6 ± 0.6†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End contractions</td>
<td>126 ± 3</td>
<td>127 ± 2</td>
<td>106 ± 4*†</td>
</tr>
<tr>
<td>End recovery</td>
<td>127 ± 3</td>
<td>129 ± 3</td>
<td>107 ± 4*†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 control rats, 6 moderate chronic heart failure (CHF) rats, and 4 severe CHF rats. MAP, mean arterial pressure. *P < 0.05 vs. control animals; †P < 0.05 vs. moderate CHF animals.

Off-transient data were compared with the on-transient (MRTon) from the same animals in which the recovery was analyzed. MRT, T63, and MAP were compared among (control vs. moderate CHF vs. severe CHF) and within (MRT and T63: on-transient vs. off-transient and MAP: end-contractions vs. end-recovery) groups with two-way ANOVAs. All other comparisons among groups were analyzed with one-way ANOVAs. Where differences were detected, a Student-Newman-Keuls post hoc test was performed. Directional hypotheses were tested via one-tailed tests, whereas all other comparisons were tested against nondirectional hypotheses via two-tailed tests. Standard linear regression and Pearson’s product-moment correlations were used to examine the relationships among central indexes of heart failure, MRToff, and PmvO2 response asymmetry (MRToff-on) when all individual animals were combined. Data are presented as means ± SE. The significance level was set at P < 0.05.

RESULTS

Body mass was not different (P > 0.05) among groups (control: 310 ± 14 g, moderate CHF: 313 ± 6 g, and severe CHF: 314 ± 16 g).

Hemodynamic and morphological indexes of heart failure. Hemodynamic and morphological indexes of heart failure are shown in Table 1. Moderate CHF rats demonstrated a significantly elevated LVEDP and RV weight-to-body weight ratio, but not lung weight-to-body weight ratio, compared with control rats. In severe CHF rats, LVEDP, RV weight-to-body weight ratio, and lung weight-to-body weight ratio were all elevated compared with both control and moderate CHF groups.

Arterial blood sampling and MAP. There were no differences in arteriolar O2 saturation (control: 93 ± 1%, moderate CHF: 95 ± 1%, and severe CHF: 95 ± 2%), systemic hematocrit (control: 41 ± 1, moderate CHF: 43 ± 2, and severe CHF: 42 ± 2), or pH (control: 7.45 ± 0.02, moderate CHF: 7.42 ± 0.02, and severe CHF: 7.46 ± 0.01) among groups (P > 0.05 for all). Within each group, there were no differences between the end-contractions and end-recovery MAP.

However, when compared among groups, the severe CHF rats was significantly lower (P < 0.05) than control and moderate CHF rats at the end of the contractions and recovery period (Table 1).

Effects of CHF on the spinotrapezius muscle PmvO2 off-transient. The effects of moderate and severe CHF on PmvO2 during recovery from contractions of the spinotrapezius muscle are shown in Table 2, and the average data from each group are shown in Fig. 1. The recovery profiles in all six control rats were well fit with a one-component model, whereas the more complex two-component model fit was indicated in the majority of CHF rats (moderate: 3 of 6 rats and severe: 3 of 4 rats). Neither r2 (control: 0.96 ± 0.01, moderate CHF: 0.96 ± 0.01, and severe CHF: 0.96 ± 0.01) nor the sum of the squared residuals (control: 20.7 ± 4.3 mmHg, moderate CHF: 28.2 ± 5.3 mmHg, and severe CHF: 18.4 ± 5.4 mmHg) of the model fits were different (P > 0.05 for both) among groups. Within each group, end-recovery PmvO2 was not significantly different (P > 0.05) from the respective precontraction baseline PmvO2. There was a significant slowing of the kinetics of the PmvO2 off-transient response (MRToff and T63) in moderate CHF rats compared with control rats, which was further exacerbated in severe CHF rats such that PmvO2 during

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Table 2. PmvO2 kinetics parameters during recovery from contractions in spinotrapezius muscles of healthy control, moderate CHF, and severe CHF rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Moderate CHF</th>
<th>Severe CHF</th>
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<tbody>
<tr>
<td>PmvO2(end-con), mmHg</td>
<td>14.4 ± 1.3</td>
<td>16.1 ± 2.3</td>
<td>10.9 ± 1.1*†</td>
</tr>
<tr>
<td>ΔPmvO2, mmHg</td>
<td>7.7 ± 1.2</td>
<td>7.5 ± 1.1</td>
<td>2.4 ± 0.3*†</td>
</tr>
<tr>
<td>ΔtPmvO2, mmHg</td>
<td>–</td>
<td>5.7 ± 1.0</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>ΔtotalPmvO2, mmHg</td>
<td>7.7 ± 1.2</td>
<td>10.4 ± 0.9</td>
<td>6.1 ± 0.4†</td>
</tr>
<tr>
<td>PmvO2(end-rec), mmHg</td>
<td>221 ± 1.8</td>
<td>265 ± 2.4</td>
<td>170.5 ± 1.5†</td>
</tr>
<tr>
<td>T1/2, s</td>
<td>3.5 ± 1.3</td>
<td>5.1 ± 2.1</td>
<td>4.1 ± 2.9</td>
</tr>
<tr>
<td>Td, s</td>
<td>–</td>
<td>71.4 ± 20</td>
<td>90.7 ± 3.7</td>
</tr>
<tr>
<td>τ1, s</td>
<td>56.9 ± 6.5</td>
<td>48.7 ± 14</td>
<td>62.8 ± 14</td>
</tr>
<tr>
<td>τ2, s</td>
<td>–</td>
<td>32.3 ± 12</td>
<td>42.9 ± 6.5</td>
</tr>
<tr>
<td>MRToff, s</td>
<td>60.2 ± 6.9</td>
<td>72.8 ± 6.6†</td>
<td>109.8 ± 6.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 control rats, 6 moderate CHF rats, and 4 severe CHF rats. PmvO2, microvascular PO2; PmvO2(end-con), end-contraction PmvO2; ΔPmvO2, amplitude of the first component; ΔtPmvO2, amplitude of the second component; ΔtotalPmvO2, overall recovery amplitude regardless of a one- or two-component model fit; PmvO2(end-rec), end-recovery PmvO2; T1/2, time delay for the first component; Td, time delay for the second component; τ1, time constant for the first component; τ2, time constant for the second component; MRToff, mean response time for the off-transient; T63, time to reach 63% of the overall recovery determined independent of modeling procedures. *P < 0.05 vs. control animals; †P < 0.05 vs. moderate CHF animals.

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(To3) was used to ensure appropriateness of the model fits. Specifically, the raw PmvO2 data were interpolated, and the time coinciding with 63% of the total recovery amplitude (ΔtPmvO2) was determined. MRToff is highly reproducible during recovery periods from repeated contractions bouts separated by 20 min (within-animal coefficient of variation: 8 ± 3 s, n = 8) with no ordering effect (overall within-animal difference: 1 ± 5 s, P > 0.05).

Data analysis. CHF rats were further divided into moderate (n = 6) or severe (n = 4) categories as described by Diederich et al. (13). Specifically, CHF rats were placed into the severe CHF category if LV end-diastolic pressure (LVEDP), RV weight-to-body weight ratio, and lung weight-to-body weight ratio were all greater than 4 SDs from the mean of control animals. All other CHF rats were placed in the moderate CHF group. Off-transient data were compared with the on-transient (MRTon) from the same animals in which the recovery was analyzed. MRT, T63, and MAP were compared among (control vs. moderate CHF vs. severe CHF) and within (MRT and T63: on-transient vs. off-transient and MAP: end-contractions vs. end-recovery) groups with two-way ANOVAs. All other comparisons among groups were analyzed with one-way ANOVAs. Where differences were detected, a Student-Newman-Keuls post hoc test was performed. Directional hypotheses were tested via one-tailed tests, whereas all other comparisons were tested against nondirectional hypotheses via two-tailed tests. Standard linear regression and Pearson’s product-moment correlations were used to examine the relationships among central indexes of heart failure, MRToff, and PmvO2 response asymmetry (MRToff-on) when all individual animals were combined. Data are presented as means ± SE. The significance level was set at P < 0.05.
recovery was slower than control and moderate CHF rats ($P < 0.05$ for both). There were significant correlations ($P < 0.01$ for all) between MRT$_{off}$ and LVEDP ($r = 0.76$; Fig. 2), RV weight-to-body weight ratio ($r = 0.74$), and lung weight-to-body weight ratio ($r = 0.79$). Importantly, the analysis and interpretation of the recovery kinetics of PmvO$_2$ and the correlations with indexes of CHF were not different whether the model-dependent (MRT$_{off}$) or model-independent ($T_{63}$) parameters were used. This observation establishes confidence in the modeling procedures used.

Effects of CHF on spinotrapezius muscle PmvO$_2$ on-off asymmetry. Significantly slower MRT$_{off}$ versus the corresponding MRT$_{on}$ (i.e., PmvO$_2$ on-off asymmetry) was observed in all groups (Fig. 3). However, the difference between MRT$_{off}$ and MRT$_{on}$ (MRT$_{off-on}$) was not different ($P > 0.05$) between control and moderate CHF animals, whereas it was increased ($P < 0.05$) in severe CHF animals compared with both control and moderate CHF animals. There were significant correlations ($P < 0.01$ for all) between MRT$_{off-on}$ and LVEDP ($r = 0.69$; Fig. 4), RV weight-to-body weight ratio ($r = 0.75$), and lung weight-to-body weight ratio ($r = 0.75$). There was no correlation ($r = -0.11$, $P = 0.70$) between MRT$_{off}$ and MRT$_{on}$.

DISCUSSION

Three principal novel findings emerged from the present investigation: 1) progressive CHF increasingly slows the recovery of PmvO$_2$ in mixed fiber-type rat skeletal muscle, 2) MRT$_{off}$ and MRT$_{off-on}$ significantly correlate with central hemodynamic (LVEDP) and morphological (RV weight-to-body weight and lung weight-to-body weight) indexes of CHF, and 3) the analysis and interpretation of the recovery kinetics of PmvO$_2$ and the correlations with indexes of CHF were not different whether the model-dependent (MRT$_{off}$) or model-independent ($T_{63}$) parameters were used. This observation establishes confidence in the modeling procedures used.
The correlations between central indexes of cardiac function and \( \text{MRT}_{\text{off}} \) and \( \text{MRT}_{\text{off-on}} \) when all animals were combined are highly novel. The present research model used submaximal electrical stimulation of a small muscle mass such that central cardiac (dys)function via cardiac output was not expected to have any direct bearing on \( \text{PmvO}_2 \) during recovery. Therefore, these correlations suggest that progressive CHF induces peripheral maladaptations that impact recovery processes negatively and occur generally in proportion to central CHF dysfunction. However, it is noteworthy that these correlations appear principally driven by the presence of the severe CHF group and may not be obligatory within groups or when healthy and moderate CHF rats are investigated in separation.

CHF has not produced consistent alterations in the on-kinetics in spinotrapezius muscle, with studies reporting a slowing (4), speeding (4,13), and/or no change (15) compared with healthy controls, and the mechanisms accounting for these observations are unclear. It is worth noting, however, that the low \( \text{PmvO}_2\text{end-con} \) and \( \text{PmvO}_2\text{end-rec} \) in severe CHF rats in the present study is consistent with lower absolute \( \text{PmvO}_2 \) values at rest and during contractions in these same rats (15) and other severe CHF populations (13). It also must be considered that, whereas clusters of aberrant on-responses are found in CHF, these vary among animals such that differences from healthy animals are blurred in the mean data. This does not appear to be true for the off-transient.

Mechanisms of microvascular dysfunction during recovery in CHF. In healthy rats, the recovery of \( \text{PmvO}_2 \) after contractions is characterized by a brief time delay in which \( \text{QO}_2 \) and \( \text{Vo}_2 \) are likely decreasing in direct proportion to one another during the recovery period. As such, any CHF-induced maladaptation that reduces postexercise blood flow relative to local \( \text{Vo}_2 \) would be expected to reduce \( \text{PmvO}_2 \) during the recovery period. Experimental evidence has demonstrated that CHF results in the attenuation (21,
30) and/or redistribution (36) of blood flow during exercise in concert with reduced Q\(\text{O}_2\) to the tissues during recovery (50). The lower postexercise Q\(\text{O}_2\) correlates with delayed metabolic (PCr) recovery (50) and is consistent with peripheral vascular dysfunction consequent to neurohumoral dysregulation (52) and reduced nitric oxide (NO) bioavailability (14). The role of reduced NO bioavailability in CHF rats is supported by the observation that in healthy rats local superfusion of arginine methyl ester (l-NAME; an inhibitor of NO synthase) slows the recovery of Pm\(\text{vO}_2\) (18), resulting in a recovery profile closely resembling that found in CHF rats. This suggests that in CHF rat spinotrapezius muscle the superfusion of l-NAME would have little or no effect on Pm\(\text{vO}_2\) recovery kinetics. Additionally, CHF slows the Pm\(\text{vO}_2\) recovery in the slow-twitch soleus but not fast-twitch peroneal muscles (34). Given that NO plays a larger role in vessels supplying predominantly slow-twitch compared with fast-twitch muscle (20, 32, 35), this observation further supports the influence of reduced NO bioavailability on altered Pm\(\text{vO}_2\) recovery dynamics in CHF.

The inability of CHF patients to effectively match Q\(\text{O}_2\) to O\(\text{2}\) demands during exercise transitions consequent to central and peripheral circulatory alterations prolongs metabolic (9, 37, 48) and central hemodynamic (38, 49) recovery. These alterations are reflected in the delayed recovery characteristic of pulmonary VO\(\text{2}\) kinetics, which correlates with exercise intolerance and reduced functional capacity (39). Additionally, CHF impairs oxidative enzyme activity (severe CHF (12)), reduces mitochondrial creatine kinase expression (16), increases the expression of inducible NO synthase (1, 16), and alters Ca\(^{2+}\) handling by the sarcoplasmic reticulum (7) within skeletal muscle. These intracellular alterations are expected to further retard metabolic recovery.

**Experimental considerations.** CHF elicits myriad complications affecting both muscle Q\(\text{O}_2\) and VO\(\text{2}\). It must be considered that CHF subjects may be markedly heterogeneous with regard to symptoms and degree of functional impairment, thus challenging research efforts. For example, as shown in Fig. 2, slower Pm\(\text{vO}_2\) recovery kinetics may not be an obligatory phenomenon in all moderate CHF rats. Notwithstanding these considerations, an important contribution of the present work is the observation that Pm\(\text{vO}_2\) off-kinetics may be slowed despite no consistent alterations in the on-transient, even in rats with the most severe heart failure (4, 13, 15). We propose that the analysis of Pm\(\text{vO}_2\) recovery kinetics in the spinotrapezius muscle of CHF rats constitutes an important research tool with which to perform mechanistic investigations into the effects of CHF on skeletal muscle microvascular dysfunction and potential therapeutic interventions.

The severe CHF group exhibited significantly reduced MAP at the end of contractions and at the end of the recovery period compared with both control and moderate CHF rats (Table 1). Our laboratory (6) has previously demonstrated that arterial hypotension does not affect Pm\(\text{vO}_2\) kinetics parameters until MAPs of <70 mmHg are reached. Therefore, we are confident that the prolonged Pm\(\text{vO}_2\) recovery dynamics evident in severe CHF rats are the direct result of the CHF-related vascular and muscle pathology per se and not secondary to the lower MAP.

**Summary and conclusions.** Consistent with our hypotheses, the present study demonstrated that progressive CHF increasingly slows the recovery of Pm\(\text{vO}_2\) after contractions in the mixed fiber-type rat spinotrapezius muscle and that the degree of slowing positively correlates with central indexes of heart failure. Moreover, the present investigation identified, for the first time, that CHF may alter the off-kinetics in rat skeletal muscle independent of consistent alterations in Pm\(\text{vO}_2\) on-kinetics. As such, a mechanistic link is suggested between altered circulatory control and the prolonged metabolic recovery after exercise in CHF patients, which is in accordance with the delayed recovery of pulmonary VO\(\text{2}\) kinetics evident in this population. Elucidation of the mechanisms underlying the slowed kinetics of pulmonary VO\(\text{2}\) recovery in CHF patients constitutes an important step in the development of pharmacological and nonpharmacological (i.e., exercise training protocols) therapies designed to mitigate the pernicious effects of this disease on exercise performance and quality of life.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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